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In the past year we have continued our investigation of the relationship between central catecholaminergic systems and the effects of stress. We have completed or made progress in three studies of the role of the noradrenergic system in biochemical and behavioral effects of stress and one study of the role of the dopaminergic system in these behavioral effects. The first noradrenergic study concerned the mechanism of a biochemical response to stress which is believed to play a role in long term stress adaptation, the activation of the immediate early gene, c-fos, in the brain. On the basis of previous data we had hypothesized that the noradrenergic system is involved in the activation of this gene in the brain by stress. In the past year we confirmed this hypothesis by showing that the c-fos mRNA and protein responses to stress could be reduced by treatment with the beta blocker, propranolol, and enhanced by the norepinephrine (NE) reuptake inhibitor, desmethylimipramine (DMI). These findings have supported a role of the noradrenergic system in adaptational phenomena. The second and third stuides concerned the role of noradrenergic processes in two behavioral effects of stress, increased anxiety and motor impairment. In the study on stress-induced anxiety, we found that blockade of beta

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receptors with propranolol potentiates the stimulatory effect of stress on anxiety in two tests of the latter, passive avoidance and defensive withdrawal. In the study on stress-induced motor impairment we showed that both propranolol and betaxolol, a selective beta-1 receptor blocker, mimic the inhibitory effect of stress on effortful motor activity in a swimming test. These findings have led to new hypotheses concerning the role of brain beta adrenoceptors in the control of anxiety and motor impairment during stress.

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Summary:
In the past year we have continued our investigation of the relationship between central catecholaminergic systems and the effects of stress. We have completed or made progress in three studies of the role of the noradrenergic system in biochemical and behavioral effects of stress and one study of the role of the dopaminergic system in these behavioral effects.

The first noradrenergic study concerned the mechanism of a biochemical response to stress which is believed to play a role in long term stress adaptation, the activation of the immediate early gene, c-fos, in the brain. On the basis of previous data we had hypothesized that the noradrenergic system is involved in the activation of this gene in the brain by stress. In the past year we confirmed this hypothesis by showing that the c-fos mRNA and protein responses to stress could be reduced by treatment with the beta blocker, propranolol, and enhanced by the norepinephrine (NE) reuptake inhibitor, desmethylimipramine (DMI). These findings have supported a role of the noradrenergic system in adaptational phenomena.

The second and third studies concerned the role of noradrenergic processes in two behavioral effects of stress, increased anxiety and motor impairment. In the study on stress-induced anxiety, we found that blockade of beta receptors with propranolol potentiates the stimulatory effect of stress on anxiety in two tests of the latter, passive avoidance and defensive withdrawal. In the study on stress-induced motor impairment we showed that both propranolol and betaxolol, a selective beta-1 receptor blocker, mimic the inhibitory effect of stress on effortful motor activity in a swimming test. These findings have

led to new hypotheses concerning the role of brain beta adrenoceptors in the control of anxiety and motor impairment during stress.

In the study on stress-induced dopaminergic changes, we have found that stress markedly potentiates the ability of low doses of the dopamine receptor blockers, fluphenazine, eticlopride and SCH23390 to produce immobility and catalepsy in mice and rats and that this change is reversed by treatment with the DA agonist, apomorphine. These findings suggest that stress produces a dysfunction of dopaminergic neurotransmission in the corpus striatum which makes animals more vulnerable to motor disruption. This change may be related to the above beta noradrenergic finding as the latter system has effects on nigrostriatal dopaminergic function.

The above findings thus indicate that both noradrenergic and dopaminergic neuronal systems play roles in the biochemical and behavioral sequelae of stress and that it may be possible to selectively reverse or prevent the latter with appropriate pharmacological methods.

1) <u>Role of noradrenergic system in central c-fos response</u>

Previous studies by the present and other investigators had shown that stress produces an increase of c-fos mRNA and protein in the brain. As this response may subserve long term adaptational changes, we undertook studies of its mechanism. We had hypothesized that the response was the result of the release of brain NE since our previous studies had shown that NE release, which is increased by stress, leads to a marked c-fos response in the brain. This past year we haved tested this hypothesis by administering prior to stress drugs that effect the noradrenergic system and analyzing c-fos mRNA and protein content of the brain by in situ hybridization and immunohistochemistry, respectively. The drugs used have included the beta adrenoceptor antagonist, propranolol, and the NE reuptake inhibitor, DMI. We have found that propranolol at 5-10 mg/kg, i.p., 30 min prior to immobilization stress produces a significant decrease of c-fos mRNA in a number of brain regions including the frontal and piriform cortex and cingulate gyrus. We have also found that administration of DMI, 10 mg/kg, i.p., potentiated the increase in c-fos mRNA and protein produced by stress in the piriform cortex and cingulate gyrus. These observations support the hypothesis that the noradrenergic system contributes ot the c-fos response to stress and may play a role in long term adaptational processes in the CNS. An abstract of this work was presented at the Society for Neuroscience 1993 meeting (Stone et al. 1993).

2) <u>Role of beta adrenoceptors in behavioral effects of stress</u>

Although beta receptors are involved in a number of biochemical reactions to stress their role in behavioral responses to stress is still unclear. It has been hypothesized, however, that these receptors may play a role in the production of anxious behavior by stress (Gorman & Dunn, 1993). Since there had been relatively little investigation of this hypothesis we initiated a series of experiments to test it. In these experiments we measured the effect

of beta receptor blockade on anxious behavior following stress. Mice were pretreated with the beta antagonist, l-propranolol (2.5-10 mg/kg, s.c.) before being subjected to immobilization stress and were then tested for anxious behavior in two situations, a passive avoidance and a defensive withdrawal test. As has been reported previously subjecting animals to stress increased anxious behavior on both of these tests (Blanchard & Blanchard, 1989; Steenbergen et al., 1989). In conflict with the above hypothesis, however, we found that propranolol increased rather than decreased anxious behavior. Drug treated stressed mice had significantly longer latencies in both the passive avoidance and defensive withdrawal tests than saline treated stressed animals. These results suggest the novel hypothesis that the noradrenergic system does not enhance anxiety but rather inhibits it during stress. As propranolol blocks both beta adrenergic and serotonergic receptors further research with more selective antagonists will be required to confirm it. A manuscript of these findings has been submitted for publication (Stone et al. Submitted a).

A second behavior that beta receptors may be involved in is motor activity during stress. We had previously shown that noradrenergic neurons have metabolic effects on dopaminergic neurons in the substantia nigra (Bing et al. Submitted). These findings along with earlier hypotheses of a motor role of NE (Stafford & Jacobs, 1990; Stone, 1970; Weiss et al., 1975) led us to investigate noradrenergic influences on motor activity during stress. To do this we subjected mice to immobilization stress in the presence or absence of treatment with propranolol, or the selective beta-1 receptor antagonist, betaxolol and measured effortful motor activity in a swimming task. In agreement with previous studies, it was found that the stress produced a significant reduction in swimming (Armario et al., 1991). Propranolol and betaxolol when given alone both produced a similar reduction in acitivity and when given with stress significantly enhanced the latter's effect. These findings suggest the new hypothesis that neurotransmission at brain beta-1 adrenoceptors is necessary for effortful motor activity and that this transmission might be insufficient during or after stress.

3) <u>Stress-induced DA dysfunction</u>

Our finding that the noradrenergic system can influence the nigrostriatal dopaminergic system led us also to examine effects of stress on dopamine-regulated motor function. It had been reported as a preliminary observation by others that stress enhances the ability of DA receptor blockers to induce motor inactivity (Snyder et al., 1985). Since this suggested a dysfunction of the striatal DA system after stress we undertook studies to replicate and extend it. Mice and rats were subjected to immobilization stress after administration of a low dose of the DA receptor blocker fluphenazine (0.0375-0.075 mg/kg) and were then tested for immobility and catalepsy. It was found that while neither the stress nor the low dose fluphenazine produced any effect the two together produced a significant degree of immobility and catalepsy. A similar result was produced with the more selective D2 and D1 blockers, eticlopride and SCH23390. That the immobility was due to a motor or motivational deficit and not the result of stress-induced freezing was shown by the fact that diazepam, 0.5 mg/kg, did not reduce but accentuated it. Apomorphine, 0.2 mg/kg, on the other hand reversed it. The above findings

suggest therefore that stress produces a dysfunction of dopaminergic neurotransmission in the corpus striatum which makes animals more vulnerable to motor or motivational disruption. A manuscript of these findings has been submitted for publication (Stone et al., Submitted b).

Publications and Submissions (12/1/92-pres.)

Stone, E.A., Manavalan, J.S., Basham, D.A. & Bing, G. Effect of yohimbine on nerve growth factor mRNA and protein levels in rat hippocampus. Neurosci. Lett. In press.

Bing, G., Zhang, Y., Watanabe, Y., McEwen, B.S. & Stone, E.A. Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra. Submitted.

Hiller, J.M., Zhang, Y., Bing, G., Gioannini, T.L., Stone, E.A. & Simon, E.J Immunohistochemical localization of mu opioid receptors in rat brain using antibodies generated against a peptide sequence present in a purified mu opioid binding protein. Submitted

Stone, E.A., Najimi, M. & Quartermain, D. Potentiation by propranolol of stress-induced changes in passive avoidance and defensive withdrawal Submitted.

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Stone, E.A. Glial cells as targets of the central noradrenergic system: An update. In: Noradrenergic Mechanisms in Parkinson's Disease, Colpaert, F.C. & Briley, M. (eds), CRC Press, Boca Raton, 1994, pp. 173-189.

Interactions (12/1/92 - pres.)

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Stone, E.A., Zhang, Y. & Bing, G. The role of the noradrenergic system in central cfos responses. Abstr. Soc. Neurosci. 19:82, 1993.

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